The epidemiology of multiple *Plasmodium falciparum* infections

11. Premunition in *Plasmodium falciparum* infection: insights from the epidemiology of multiple infections

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Abstract

Epidemiological studies of multiple clone infections by *Plasmodium falciparum* in highly endemic areas have demonstrated age dependence in both the multiplicity of infection and the relationships between this multiplicity and the risk of acute illness. We hypothesize that, in infants, host defence against blood-stage infections with *P. falciparum* relies mainly on fever and cytokine activities, and the infections are of short duration. In older children, a high multiplicity of infection is characteristic of low-level chronic parasitaemia. This appears to confer cross-protection against newly inoculated parasites, via partially genotype-specific responses which are short-term, lasting little longer than the infections themselves. This has important implications for our understanding of immunity against *P. falciparum*, its ecological niche, and the epidemiological impact of interventions against it.

Keywords: malaria, *Plasmodium falciparum*, multiple infection, premunition, tolerance, children, Tanzania

Introduction

For the first few months of life, a child in sub-saharan Africa is protected from malaria morbidity: infections are of low density and do not last long (KITUA *et al.*, 1996). This control of infections is probably a consequence of maternal malaria-specific immunoglobulin G antibodies acquired across the placenta (EDOZIEN *et al.*, 1962; MCGREGOR, 1964), or of an effect of fetal haemoglobin (PASVOL *et al.*, 1977). However, this ascendency of the child's defences is only temporary.

In areas of the highest transmission of malaria, entomological inoculation rates (EIRs) reach several hundred infective bites per year. In these areas the terms of the child's engagement with the parasite change dramatically in the middle of its first year. It is at this age, when the haemoglobin levels are at a minimum (KITUA *et al.*, 1997), that blood-stage infections have the highest parasite densities (KITUA *et al.*, 1996), often leading to fever. The incidence of severe malarial anaemia (GREENBERG *et al.*, 1989; SNOW *et al.*, 1994) and of malaria mortality also then reach maxima. Although cerebral malaria is relatively uncommon in areas with very high EIRs (SNOW *et al.*, 1994), the anaemia may kill the child, handing a Pyrrhic victory to the parasite, but more usually the host survives.

Clinical malaria attacks then become gradually less frequent as the child grows older, and parasite densities in between the attacks steadily decrease. Although the child now seems to be gaining the upper hand, he or she remains parasitized. In fact, the prevalence of parasitaemia is highest in children older than those who are at greatest risk of clinical attacks (BRUCE-CHWATT, 1963; MOLINEAUX & GRAMICCIA, 1980; TRAPE *et al.*, 1994; SMITH *et al.*, 1999a) (Fig. 1). These children have acquired immunity which is able to control malaria parasitaemia at a low density, but does not completely eliminate the infection.

In this paper we consider the processes involved in establishing such chronic infections and their significance for the host. We do not discuss the immunological mechanisms in detail, nor do we discuss how these mechanisms depend quantitatively on the exposure of the host to malaria parasites. Our approach is to synthesize the results of studies of how malariological indices in areas of very high transmission vary with age and, especially, of recent research in molecular epidemiology. We then suggest a novel hypothesis for the role of antigenic diversity in protective immunity.

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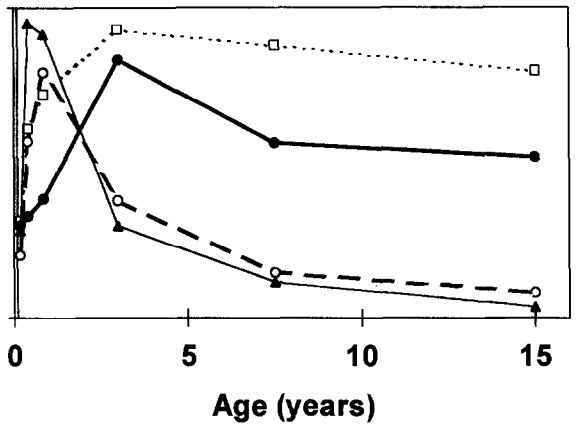

Fig. 1. Prevalence of *Plasmodium falciparum* parasitaemia (□), multiplicity of infection (●), geometric mean parasite density (○) and risk of malaria fever (▲) according to age; Kilombero Valley, Tanzania. Data from SMITH *et al.* (1999a); the top of the vertical axis corresponds to a parasite prevalence of 100%, prevalence of malaria fever of 6%, mean multiplicity of 6·0, and geometric mean parasite density of 6000 parasites/μL.

Mechanisms of chronic infection by *Plasmodium falciparum*

The mechanisms which enable the partially-immune host to control chronic infections without eliminating them altogether must be very potent, but nevertheless exhibit only an incomplete and non-sterilizing antiparasitic action (DRUILHE & PÉRIGNON, 1997). One theory is that antibody responses to the cytoadherence ligand *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) account for the persistence of infections. PfEMP-1 undergoes clonal antigenic switching, with its antigenic type changing at a high frequency during the course of the intra-erythrocytic cycle (BIGGS *et al.*, 1991; ROBERTS *et al.*, 1992). This variation has been proposed to be the explanation of chronic infections: as the host develops a specific antibody response to one antigenic type, switching leads to new types which are able to escape the existing repertoire of host responses and multiply. However, DRUILHE & PÉRIGNON (1997) contended that such a system would result in much wider fluctuations in parasite densities than those observed in partially-immune adults. They have proposed that an indirect, monocyte-dependent mechanism regulates parasite densities independently of their antigenic type.

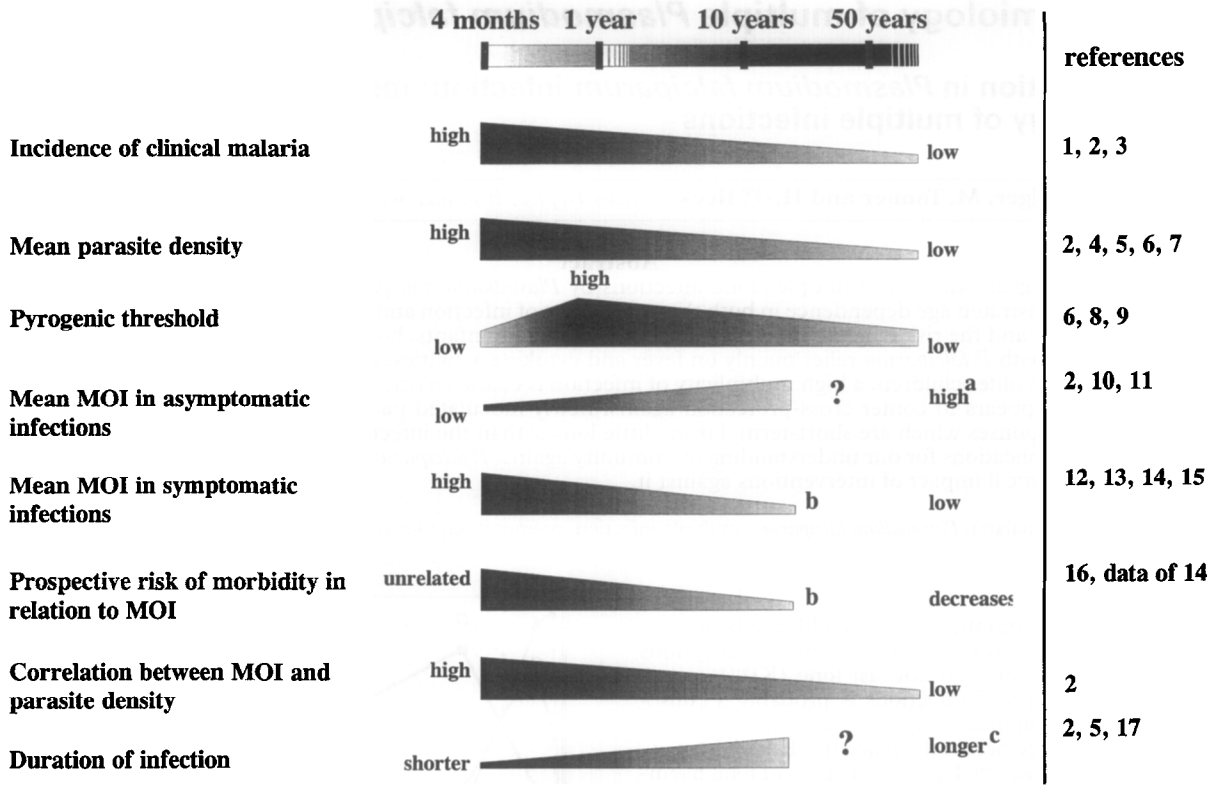

Fig. 2. *Plasmodium falciparum* malaria in highly endemic areas: summary of age dependence of key factors related to multiplicity of infection (MOI); **a** indicates that the lower MOI observed in older individuals may have been due to reduced sensitivity at very low densities; **b** indicates that data for adults on the mean MOI in symptomatic infections and the prospective risk of morbidity in relation to MOI were not available due to the low frequency of clinical episodes; **c** indicates that the short durations of infection in adults, estimated from microscopy data, may have been due to reduced sensitivity of microscopy at low parasite densities. References: 1, TRAPE *et al.*, 1994 (Senegal); 2, SMITH *et al.*, 1999a (Tanzania); 3, SMITH *et al.*, 1995 (Tanzania); 4, MOLINEAUX & GRAMICCIA, 1980 (Nigeria); 5, GENTON *et al.*, 1995 (Papua New Guinea); 6, ROGIER *et al.*, 1996 (Senegal); 7, SMITH *et al.*, 1993 (Tanzania); 8, SMITH *et al.*, 1994 (Papua New Guinea); 9, ROOTH & BJÖRKMAN, 1992 (Tanzania); 10, NTOUMI *et al.*, 1995 (Senegal); 11, KONATÉ *et al.*, 1999 (Senegal); 12, ROBERT *et al.*, 1995 (Senegal); 13, BECK *et al.*, 1997 (Tanzania); 14, FELGER *et al.*, 1999 (Tanzania); 15, SMITH *et al.*, 1999b (Tanzania); 16, AL-YAMAN *et al.*, 1997 (Papua New Guinea); 17, BEKESSY *et al.*, 1976 (Nigeria).

The mechanisms which regulate the parasite density are not the only ones required if chronic malaria infection is to occur. The chronically parasitized host must also be able to remain infected with *P. falciparum* without symptoms of acute disease. This malaria tolerance* is currently believed to be mediated by the host response to malaria toxins (CLARK *et al.*, 1997). These are different mechanisms from those which control parasite densities. When tolerance is assessed epidemiologically, it is found that the age dependence of tolerance differs from that of the control of density. Adults have less tolerance of high parasitaemia than have all but the youngest children (SMITH *et al.*, 1994; ROGIER *et al.*, 1996).

Chronic infection will itself induce immune responses, which in turn might influence any of several malariological outcome measures: (i) the risk or rate of reinfection; (ii) parasite density; (iii) the incidence of clinical malaria; and (iv) the severity of disease.

Multiplicity: an indicator of chronic infection

The availability of polymerase chain reaction (PCR) techniques for genotyping parasites has added further outcome measures: the specific genotypes of *P. falciparum* identifiable within a host and the number of co-infecting parasite genotypes, or multiplicity of infection†. Studies in Senegal, Papua New Guinea and Tanzania have shown that children can be infected with at least 9 different clones‡ of parasites at once (see the references cites in the legend to Fig. 2) and that the multiplicity varies with age (Fig. 1). Rather than trying to dissect the relationships between specific immune responses and the genotypes of individual infections, in this paper we pursued an epidemiological approach and assessed the significance of infection multiplicity by reviewing evidence from molecular epidemiological field studies in Papua New Guinea, southern Tanzania and Senegal.

One might suppose that multiplicity of infection merely reflects exposure intensity. If this were the case, transmission-reducing measures, such as use of insecticide-impregnated bed nets, should reduce multiplicity in proportion to the effect on transmission. One would also expect (on the assumption that each infection behaves independently) that both parasite densities and morbidity rates would be proportional to multiplicity. Indeed, this is what is observed in infants after the protection conferred by maternal factors wanes. However, in older children, these malariological indices show

*Tolerance in this context is the ability of the partially-immune host to remain infected with *P. falciparum* without symptoms of acute disease. Tolerance can be assessed epidemiologically by estimating the average parasite density at which symptoms begin (the fever threshold; see, e.g., ROGIER *et al.*, 1996) or the probability of acute illness as a function of parasite density (SMITH *et al.*, 1994).

†By multiplicity we mean the number of distinct clones identified using any specific analytical scheme. This represents a minimal estimate of the actual number of clones circulating in an individual, which will generally be higher than the number identified (CONTAMIN *et al.*, 1995).

‡'Clone' or 'infection' refers to those blood-stage parasites descended from a single product of meiosis in the mosquito. In highly endemic areas cross-mating is frequent (BABIKER *et al.*, 1995), and so most inoculations probably consist of several different clones.

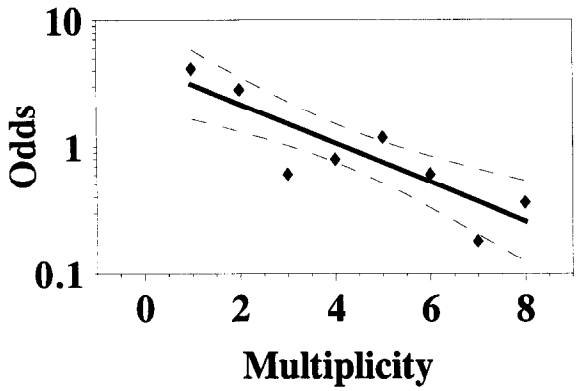

Fig. 3. Odds of clinical malaria attacks in 2–7 years old children according to multiplicity of *Plasmodium falciparum* infection (rural Kilombero district, Tanzania; after BECK *et al.*, 1997). Broken lines indicate 95% confidence intervals.

quite different relationships (Figs 2, 3).

The first puzzling finding is that the average multiplicity of infections increases at the same time as children acquire immunity and the ability to control parasite densities below the pyrogenic threshold. This is mainly because the average duration of infections increases (SMITH *et al.*, 1999a) even though the children are becoming more immune. Infections in the 6–12 months' age group last longer than those in new-born infants, but those in older children last even longer (SMITH *et al.*, 1999a, 1999b). The main defences of the 6–12 months' age group are presumably fever and related cytokines associated with clinical malaria, which are known to have a strong antiparasitic effect (KWIATKOWSKI, 1991). This strategy of host response tends to clear infections rather than to allow them to continue at low densities. As the children age, however, mechanisms which allow persistence of chronic infections become more important. Consequently, the average multiplicity in children is a good indicator of the extent of chronic parasitaemia, and thus of their immune status.

In adults, average parasite densities are very low (Fig. 1). The trends for infection duration to increase with age, and for multiplicity to become less correlated with density as the host ages, also continue into adulthood (SMITH *et al.*, 1999a). Measured multiplicity of infection in adults, however, is lower than in older children and adolescents (NTOUMI *et al.*, 1995; KONATÉ *et al.*, 1999; SMITH *et al.*, 1999a). However, this may be an artefact associated with the very low parasite densities, which might fall below the detection threshold of PCR.

Chronic infections protect against superinfections

Among older age groups it is now firmly established that patients with clinical malaria have a lower average multiplicity of infection than asymptomatic children from the same community. This was shown clearly in the Kilombero (BECK *et al.*, 1997) (Fig. 3) and Rufiji districts (FÄRNERT *et al.*, 1997) of Tanzania and in Senegal (ROBERT *et al.*, 1996). The same tendency was evident in the data of ENGELBRECHT *et al.* (1995) from Papua New Guinea. ROBERT *et al.* (1996) also found that severe malaria patients in Dakar had lower multiplicity than mild cases.

There are 3 possible explanations for the reduced multiplicity of infection in clinical cases. (i) The effect might be an artefact caused by single dominant clones in clinical cases diluting the PCR template of low-density co-infections. At high ratios of template, rare clones are not amplified (CONTAMIN *et al.*, 1995). (ii) The reduced multiplicity in clinical cases might be a consequence of the antiparasitic effect of the cytokines released during fever, or of fever itself. (iii) There is evidence from both molecular typing (CONTAMIN *et al.*, 1996) and patterns of seasonality in morbidity (LINES & ARMSTRONG, 1992) that clinical malaria is normally caused by newly invading parasites. Hence, it may be that individuals with fewer concurrent infections are more vulnerable to clinical malaria because they have a limited repertoire of cross-protection provided by clones which are already present.

If hypotheses (i) and (ii) were the only mechanisms operating, the effect should be observed irrespective of the immune status of the host. However, the effect was not seen in infants (FELGER *et al.*, 1999) or in recipients of the SPf66 malaria vaccine (BECK *et al.*, 1997).

Hypothesis (iii) predicts that the reduced multiplicity should not only be observed at the time of the clinical episode and afterwards, but also that high multiplicity in cross-sectional surveys should be associated with subsequent protection.

In fact, in a prospective study in Papua New Guinea the presence of multiple infections in individuals during cross-sectional surveys was actually associated with subsequent protection (AL- YAMAN *et al.*, 1997). It thus seems likely that in older children established infections do offer cross-protection against invading clones, either by preventing superinfecting clones from becoming established or via tolerance of the new infections.

The idea that existing chronic infections protect against superinfection dates back to SERGENT & PARROT (1935) who coined the term *Prémunition* for *'l'état particulier de résistance (aux superinfections), contemporain de l'infection et cessant avec elle'*.

Premunition has since come to refer in common malariological usage to something logically distinct from this. Thus, for instance, COHEN & DEANS (1988) used the world premunition to refer to 'acquired immunity [which] most frequently controls, but does not eliminate, the infection...'. In this meaning of premunition, it is synonymous with processes leading to low-level chronic parasitaemia. However, in the original sense of the term, which we use in this paper, the outcome of premunition (concomitant immunity) is limitation of superinfections: this is not mentioned by COHEN & DEANS (1988).

These 2 meanings can easily be confused, since premunition in the original sense cannot occur unless chronic infection can be established. This in turn requires that the individual also mounts an immune response which controls parasite densities sufficiently to prevent clinical attacks but allows the original infection to remain at low density (as proposed, for example, by DRUILHE & PÉRIGNON, 1997). If the host response is such that it eliminates all the parasites, pre-existing infections will not be able to persist long enough for such protective immune responses to develop. Similarly, if there is inadequate tolerance of parasitaemia infections cannot persist.

This conceptual framework makes it clear why young children (infants) who have lost their maternal immunity are extremely vulnerable to clinical malaria. Such children have not yet developed either tolerance or the capacity to control parasite densities, and concomitant immunity is therefore also absent since the preconditions for persistent infections do not exist.

The role of antigenic diversity

Although some blood-stage malaria antigens are highly conserved (e.g., the ring-infected erythrocyte surface antigen, RESA), others present a bewildering medley of different epitopes to the immune system. In particular, many antigens expressed during the erythrocytic cycle (S-antigen, and the merozoite surface proteins MSP 1 and MSP 2) show a high degree of polymorphic diversity within parasite populations (ANDERS, 1991; KEMP, 1992). For instance, 58 different non-synonymous genotypes were found at the *msp2* locus of *P. falciparum* in 1034 samples from children attending an outpatient clinic in Tanzania (IRION *et al.*,

1998). Unlike variation in PfEMP-1, allelic diversity in merozoite surface antigens arises as a result of meiotic recombination in the mosquito (KERR *et al.*, 1994). Both comparisons of rates synonymous with non-synonymous substitutions in the deoxyribonucleic acid sequences (HUGHES & HUGHES, 1995; FELGER *et al.*, 1997) and population genetic analyses of allele frequency data (CONWAY, 1997) indicated that most of this diversity is maintained by natural selection. It has been thought that this immense diversity has a role in exhausting the immune system in order to protect active sites of the molecules (ANDERS & SMYTHE, 1989).

In the context of this diversity, it is pertinent to ask whether immune responses are specific for homologous haplotypes only, or whether they also recognize heterologous parasites.

The control of parasite densities in older children appears to be only partly genotype-specific. Part of the evidence for this is that the density of parasitaemia is not proportional to the multiplicity of infection (Fig. 4). If the density contributed by each clone of parasites were regulated only by haplotype-specific immune responses independent of those controlling other clones, then on average the arithmetic mean density would be proportional to the number of clones circulating. In infants, density does increase with multiplicity in this way (Fig. 4), but in older individuals such a pattern is not seen.

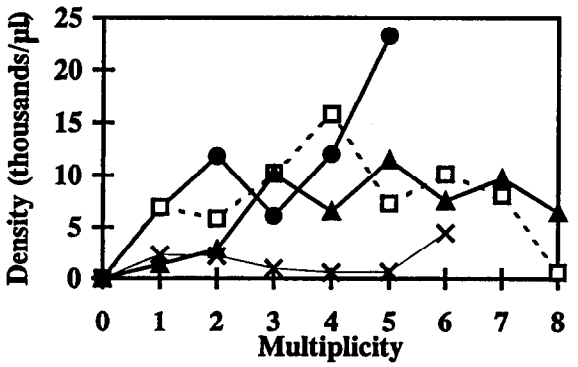

Fig. 4. Arithmetic mean *Plasmodium falciparum* parasite density according to multiplicity of infection at ages <12 months (●, FELGER *et al.*, 1999), 0·5–2·5 years (□, FRASER-HURT *et al.*, 1999), 2–7 years (▲, BECK *et al.*, 1997) and >10 years (×, SMITH *et al.*, 1999a).

Parasite densities in asymptomatic individuals also do not vary in proportion to seasonal changes in transmission intensity (SMITH *et al.*, 1993), again suggesting that different clones in the same individual do not behave independently and that the overall density is being regulated by mechanisms which do not completely discriminate between the progeny of different inoculations.

Nevertheless premunition must involve some degree of specificity. If the protection against incoming parasites were completely independent of genotype, only the presence or density of existing infections, not the extent of antigenic diversity among them, would affect the degree of protection. The reduction in risk associated with high multiplicity (Fig. 3) therefore implies some haplotype specificity, although this specificity is not necessarily conferred by the same polymorphic genes as were typed to determine multiplicity.

On the other hand, premunition cannot be highly specific for the parasite's haplotype or antigenic make-up if highly polymorphic antigens are involved in protection. Because any combination of alleles of several or many antigens might occur in nature, clones homologous for all polymorphic antigens of existing infections will form only a very small proportion of superinfections. Consequently, if protection were against homologous infections only it would be very limited and thus undetectable. If protective immune responses are indeed directed against a whole array of polymorphic antigens, then the relevant immune responses must offer some degree of cross-protection against heterologous superinfection.

Premunition does not, however, preclude other, age-dependent and haplotype-independent effects on parasite densities, which either might be gradually acquired in response to exposure or might reflect the degree of maturity of the immune system (BAIRD *et al.*, 1991). Nevertheless we hypothesize that the outcome of infections is substantially determined by immune responses directed towards the particular antigenic make-up of concurrent or recent infections. It is known that responses against merozoite antigens are rather short-lived (FRÜH *et al.*, 1991; FERRANTE & RZEPCZYK, in press). We propose that such responses contribute to the control of parasite density, possibly partly as suggested by DRUILHE & PÉRIGNON (1997). They also confer cross-protection of a variable degree against heterologous infection, dependent on the antigenic relatedness of the newly infecting haplotype. These responses to current infections might affect the outcome of superinfection by restricting the rate of newly infecting haplotypes which could become established in the host. Parasites which have a similar antigenic make-up to those already present would be excluded or controlled to a degree reflecting the relatedness of their antigenic haplotypes. Therefore superinfections which shared protective epitopes recognized by pre-existing immune

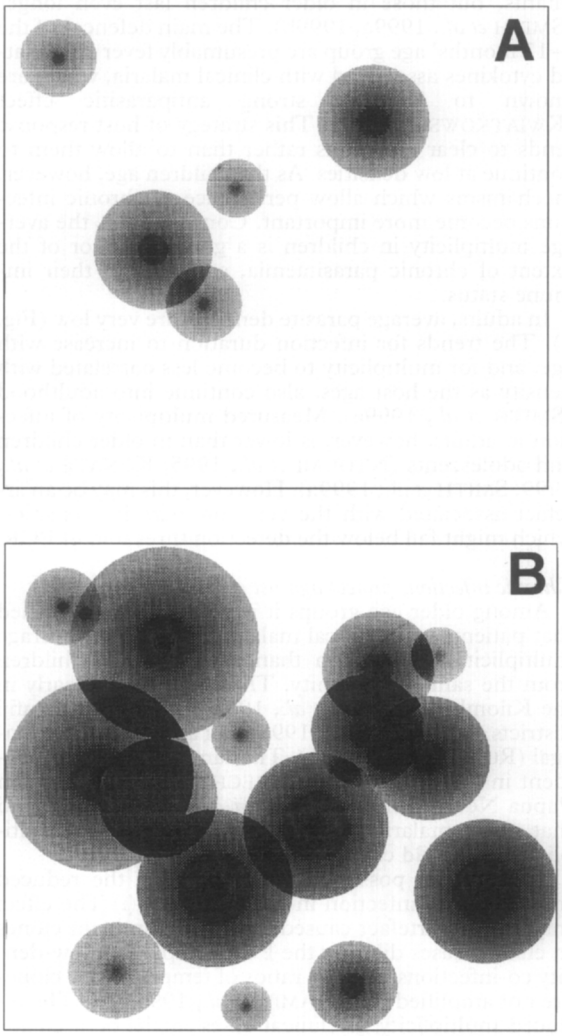

Fig. 5. Schematic illustration of malaria premunition hypothesis: A, infants and B, children 2–7 years old. See text for further explanation.

responses would be controlled and would not cause morbidity.

Our hypothesis is illustrated schematically in Fig. 5. Each frame (A and B) represents a hypothetical immunological space comprising the total possible repertoire of immune responses within one host. The centres of the circles correspond to the immunological specificities of the haplotypes currently infecting the host. The sizes and intensities of the circles are determined by the time since the infection with that haplotype began and by the immunogenicity and persistence of the responses against its epitopes. The intensity of shading then portrays the degree of cross-protection: the darker the area on to which an incoming infection maps, the more the host is protected against it.

The 2 frames illustrate how our hypothesis explains the dramatic differences between very young children and older people. In infants or other susceptible individuals, because of the non-specificity of fever responses including cytokine action, infecting haplotypes are eliminated quickly, preventing the establishment of chronicity (Fig. 5, A). In older children in endemic areas the immunological space is already largely occupied and new haplotypes cannot get established (Fig. 5, B).

The role of antigenic diversity in this model, in which existing infections contribute substantially to protection, contrasts with the theory that immunity is strictly strain-specific and long-term (GUPTA & DAY, 1994; GUPTA *et al.*, 1994). Moreover, these findings also have important consequences for our understanding of the evolution and ecology of *P. falciparum*, which are currently the subject of a series of other studies.

Parasite genotyping has enabled us to gain some understanding of premunition in *P. falciparum* but there is much still to be learnt about both immunological aspects of chronic infection and its practical implications for malaria control programmes. Our hypothesis provides a straightforward explanation for the short-term rebounds in morbidity rates observed when children are removed from antimalarial prophylaxis (GREENWOOD *et al.*, 1995; MENENDEZ *et al.*, 1997). We have also considered the implications of premunition for both long-term and short-term effects of impregnated bed nets (FRASER-HURT *et al.*, 1999; SMITH *et al.*, 1999c) and for the impact of asexual-stage malaria vaccines (SMITH, 1998). Premunition is clearly central to any comprehensive understanding of naturally acquired immunity to malaria.

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